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Pathogenicity and Virulence: Another View†

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INTRODUCTION

A modern clinical microbiologist who asks what is a pathogen and what is meant by virulence will meet with derision at best and will probably be declared a heretic, bereft of his or her senses. After all, more than 100 years have passed since Pasteur and Koch clearly demonstrated the relationship between the microbial world and disease. Two of the best available textbooks of medical microbiology, for example (21, 25), state that a pathogen is a member of a microbial species and that virulence defines the specially harmful propensities of strains within such a pathogenic species. These definitions have directed the attention of microbiologists, physicians, veterinarians, and plant pathologists to a select number of microorganisms and viruses obviously involved in causing harm to higher forms of life. Once recognized, ingenious methods were invented to curtail harmful microbial activities in a specific host and to prevent the spread of such organisms from one individual to another. These activities resulted in the invention of chemotherapeutic and antimicrobial agents, the most recent success in our fight against microbial incursions. But we must also not forget that vaccines, sterilants, and sterilizing procedures, disinfectants, antiseptics, immunoglobulin therapy, the concepts of epidemiology and immunology, and the many developments within the discipline of microbiology originated as a consequence of attempts to stem infectious disease. In view of these successful advances against infectious scourges of humanity, especially in the developed parts of the world, doubt about the meaning of pathogenicity and virulence seems inappropriate, if not ridiculous.

But the microbial world, renowned for its refusal to read the literature and its unwillingness to obey the dictates of the lords and ladies of creation, has demonstrated with increasing frequency that there are exceptions. A growing number of carefully designed antimicrobial agents does not prevent hospital-acquired infections. These infections are not the result of established pathogens endowed with special virulence attributes. Instead, they are caused by microorganisms, widely distributed in nature and without any property or principle that would signify potential harm to patients. Many of these microorganisms resist antimicrobial agents and they complicate the recovery of patients whose immunity has been embarrassed by disease or therapy; their ubiquity in nature usually does not lead to disease in healthy residents in the community. Many of these forms are rarely, if ever, even members of the intimate biosphere of the healthy population. But in the hospital setting, these bacteria and fungi (we know next to nothing about viruses and protozoa in this setting) become involved in infectious disease, especially of those patients who have benefited the most from medical science. Nosocomial disease, legionellosis, and the infectious complications of acquired immune deficiency syndrome illustrate why the pathogenicity and virulence concepts are not sufficient to explain fully the

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harmful interactions between the microbial world and the human host.

The need to readjust our perception of infection and especially of the role played by microorganisms is not specific to microbiology and infectious disease. Indeed, the recent concept of disease in general has shifted from the classical theory to one that emphasizes ecology (13). The former maintains that each disease has "one and only one cause" and that function is linked to structure. A change in function should then result in a change in structure and vice versa. In opposition to this approach, a much broader view now prevails that invokes environmental and social factors as significant contributors to the appearance and outcome of a disease process. An adjunct to this latter perception of disease, made popular by Dubos (26, 27), is our acceptance of polymicrobial infections as no longer the exclusive domain of anaerobic bacteria.

Discussion of pathogenicity requires a definition of infection as used in this essay. *Dorland's Illustrated Medical Dictionary* (3) defines infection as "the process in which pathogenic organisms invade the tissue or organs of the body and cause injury followed by reactive phenomena." Others (70) use the term to describe the presence of organisms on or in the host, a situation often equated with colonization. I shall use the term infection to describe the entire spectrum of conditions encompassed by the term infectious disease. We must remember that infection is a progressive process (42) that results eventually in obvious signs and symptoms of disease accompanied by pathological changes in the host. There are some phases of infections that are not readily apparent. Simon (70) described attenuated infection as a condition caused by potentially harmful organisms not followed by overt clinical disease. Obviously, the carrier state characteristic of the recovery phase of many infections falls within this definition, as does the prodromal or incubation stage. To this consideration we must add microbial persistence (57), the continued presence of microorganisms in host tissues after adequate therapy and cessation of obvious illness. At times, persistence may lead to relapse, but disease may not become apparent despite our ability to isolate the causative agent from tissue specimens. Simon (70) also described latent infection as the presence of microorganisms in or on tissues in low numbers. These organisms seem to differ physiologically and biochemically from variants involved in progressive disease. Their presence is detected only when suitable indicator hosts are used. Obviously, infection includes an entire spectrum of expressions that varies from the inapparent to fulminating manifestations, from acute to chronic, from self-terminating to life-threatening. Which aspect of this panorama shall be expressed in any particular individual remains unpredictable. This inability to characterize a particular response in any chosen individual has stimulated this reexamination of the concepts of pathogenicity and virulence. The experience of clinical microbiologists also supports the need for such a reexamination. Working at the bench, they have encountered microorganisms visible on stained preparations that defied all efforts of cultivation. We have isolated and identified organisms that we knew were harmless in one type of specimen but were involved in pathology when recovered from a different source. Many microorganisms, known members of the normal human microbiota, have complicated underlying diseases of patients or led to disease in nonimmune contacts. Repeated laboratory subcultures of microorganisms resulted in the loss of capsules or other attributes noted originally. These observations raise questions about the roles of these microbial characters in the production of patients' symptoms and, perhaps more important, have troubled microbiologists about the relationship between the laboratory isolate and the microorganisms in vivo.

The many microorganisms considered harmless yet manifest as etiological agents in a disease process and as isolates in the clinical laboratory reinforce the need to question the prevailing attitudes toward pathogenicity and virulence.

PATHOGENICITY: KOCH'S POSTULATES

The classic concepts of pathogenicity and virulence have provided a successful model for analyzing the role of microorganisms in disease production. We tend to overlook the fact that the explanations for even scientific phenomena are tinged by the prevailing attitudes of society at large. The late 19th century was enthralled by the concepts of Herbert Spencer and his dictum of the "survival of the fittest." Many aspects of human endeavor were influenced profoundly by this attitude, including economics (Marx), biology (Darwin), and psychology (Freud). The giants of microbiology, Pasteur and Koch, did not escape this influence, which culminated in the guiding dogma of infectious disease, Koch's postulates (42). We forget that Koch's efforts were directed at a scientific definition of the relationship between microorganisms and disease at a time when the medical and scientific communities denied this role. His masterpiece, *Die Aetiologie der Tuberkulose* (48), was clearly intended to persuade colleagues that *Mycobacterium tuberculosis* could produce all of the observed symptoms and signs of the disease. Unfortunately, his disciples have elevated this hypothesis to a quasilegal status despite the growing evidence that the postulates define infectivity rather than pathogenicity. Koch did not state his concept in the manner usually quoted. These postulates are rendered best in the latest edition of Topley and Wilson (85): (i) the organism should be found in all patients with the disease in question and its distribution in the body should correspond to the lesions observed; (ii) the organism should be cultivated outside the body of the host in pure culture for several generations; (iii) the organism so isolated should reproduce disease in other animals. The present editors of Topley and Wilson (85) emphasize the importance of specific antibody rise during recovery as an important adjunct to Koch's postulates. H. Smith (71–77), a leading investigator of human infectious disease, supports Koch's dicta, as do most past and present followers of Koch. No one can deny the profound effect of this approach. It resulted, as stated earlier, in a successful curtailment of most major infectious diseases in developed countries. However, even this success was helped substantially by the efforts of 19th century sanitary engineers who provided the means for safe drinking water and appropriate waste disposal. A careful study of Koch's efforts reveals several problems.

LIMITATIONS OF LABORATORY METHODS

In the laboratory, we have failed to cultivate many microorganisms present in or on the human body, even some highly suspected to be etiological agents of disease. The early microbiologists demonstrated the need for pure cultures to identify etiological microorganisms and establish their role in disease production. We cannot deny that the dogma of the pure-culture technique, still required in today's microbiology laboratory, forces us to deal with the microbial world under the most unnatural conditions (20). We separate each organism from companions of the same and many other

species; we provide them with an environment rich in nutrients and free of microbial and host control factors. We forget that this luxury is not known to exist in nature but base our experimental evaluation of potential pathogenicity on laboratory observation and use only laboratory-grown strains to study pathogenicity. Despite our success in cultivating an appreciable number of microorganisms, there are considerable gaps in our ability to cultivate all microorganisms seen in a stained preparation of certain specimens. While many important clinical specimens contain only one organism, a Gram-stained sputum or stool specimen teems with every imaginable morphotype demonstrating gram-positive, gram-negative, and gram-variable forms. Dworkin (28) has explained that various developmental stages of bacteria and other microorganisms expressed in the natural setting may not become apparent under laboratory conditions that usually single out select phases. However, even this form variation does not account for all of the organisms seen on smears that are not isolated on the various media at our disposal. Several tinctorial methods, including the use of fluorescein-tagged antibodies and (in the future) molecular probes, reveal that microorganisms resistant to laboratory domestication are present. I do not intend to ascribe any host-deleterious effect to such organisms. They may or may not have a role in a disease equation. Certainly, our ability to detect viruses in this setting is in its infancy at best. In view of the ecological concept of disease, the role of viruses in the initiation of host-damaging processes followed by bacterial aggravation of the damage is a possibility worthy of consideration. But we do not know the beneficial or harmful role played by a large number of microorganisms in intimate association with exposed body surfaces. Since recovery from blood and other usually sterile body fluids is a cardinal sign of pathogenicity and virulence, our inability to recover these "unknown" organisms from such specimens is advanced as an argument against their clinical significance. However, inability to culture and isolate these forms makes such an argument specious.

ANIMAL STUDIES VERSUS NATURAL DISEASE

Koch's postulates ignore the portal of entry and the ecological niche of a specific microorganism, two separate but interrelated considerations. The effort to demonstrate pathogenicity by disease production in experimental animals involves the unnatural introduction of a laboratory-grown representative, usually by intravenous, intraperitoneal, intradermal, and subcutaneous injections. Often, several animal species are required to demonstrate Koch's intent, and certain agents obviously involved in human disease, such as *Neisseria gonorrhoeae* and *Treponema pallidum*, have not found appropriate animal hosts in which to demonstrate their harmfulness. The experimental inoculation routes do not mimic the entry of the organism under usual conditions; they bypass an entire array of host and microbial processes that distort completely most natural sequences. The classical experiments with the pneumococcus and laboratory mice underline the need to use the usual portal of entry for assessing pathogenicity. Mice do not respond to encapsulated aerosolized pneumococci, while intraperitoneal injections of these bacteria invariably are lethal, to cite just one example of a challenge to Koch's accepted doctrine. Similarly, many members of the endogenous microbiota that are obviously harmless when in their usual ecological niche can produce disease in another anatomic site. *Escherichia coli* is an expected constituent in the colon or in feces. However, in extracolonic organs and tissues, *E. coli* certainly is involved in overt disease production. Under very special and comparatively rare circumstances, this bacterium acquires the capability to express disease proclivities in the colon.

Unfortunately, our collective ignorance permits only the suggestion that certain genetically controlled, possibly plasmid-associated factors may be responsible for this conversion to harmfulness. These observations raise another important, consistently neglected consideration of the human endogenous microbiota. Their number exceeds that of the cells that constitute our bodies (65); surely, microorganisms must influence our well-being as profoundly as they effect disease. Unfortunately, our preoccupation with the microbial world has been almost exclusively limited to its role in human disease, spoilage, contamination or destruction of our food supply, and the integrity of our manufactured goods (41). We have exploited microbial activities to our benefit without gaining a real understanding of the decisive role of microorganisms in making life on this planet possible (84). This lack of knowledge profoundly hinders our efforts to understand the disturbance in our microbiota that converts a commensal, potentially beneficial organism into an adversary.

INFECTIVE DOSE

Koch's postulates do not consider the number of organisms required to initiate infection, the minimal infective number. Does one representative suffice, or does a disease process require many more? In practical terms, does the isolation of a single representative or species deemed pathogenic indicate the presence of disease? When one considers the many vagaries that attend the procurement, transport, and laboratory handling of specimens, the temptation to reply in the affirmative is great, but the chance discovery of a potentially harmful organism in the absence of clinical symptoms at best leads to further laboratory isolation efforts and cautious patient observation. The minimal infective number of established etiological agents varies greatly, ranging from very few for *Shigella* spp. to many thousands for most. The studies that led to these conclusions (85) reflect the number of organisms in the lesions at the time of obvious infection. There is little or no information about the numbers to which patients are exposed originally or about the conditions that allow the original inoculum to attain minimal infective numbers. Certainly the mere presence of an organism cannot be equated with disease in most cases; historical information and the immune status of each patient are required for interpretation. Also, there is no guarantee that a nonimmune healthy host shall always progress to full-blown disease. The admission that these events are still shrouded in mystery does not excuse Koch's failure to consider this aspect.

POLYMICROBIAL AND NOSOCOMIAL INFECTIONS

Polymicrobial and nosocomial infections seldom comply with Koch's principles. Polymicrobial infections have not as yet been fully explained; they depend on not only specific host conditions, but also microbial interactions. Our preoccupation with pure-culture methodology has distorted our perception of events occurring in nature. In most instances, microorganisms are dependent on one another's biochemical activities, composting complex substrates sequentially (11). We can assume that this interdependence pertains for the activities of the normal microbiota. Possibly, the role of

viruses as initiators of host damage facilitates participation by the normal microbiota and extends host injury. However, very little is known of viruses as colonizers of body surfaces. Aside from this scenario, trauma to and pathology of tissues with or close to colonized surfaces can be followed by progressive infective activities by normal microbiota. Nosocomial infections, on the other hand, may result from microbial residents of medical facilities or from endogenous organisms. These organisms are selected by antibiotic regimens that curtail growth or survival of certain endogenous microbiota. The significance of polymicrobial and nosocomial infections with respect to Koch's postulates is the failure of these rules to explain these infections. In the final analysis, Koch's principles place the onus of disease production squarely and solely on the microorganism. They ignore the host's role in the total progression of infectious manifestations. They fail to consider as well other microorganisms in the host biosphere that may modulate the disease-associated tendencies of many microorganisms. In addition, the host's experience, environment, and risk factors not directly related to a particular health problem are overlooked. The complex interactions of a host with endogenous and exogenous microbiota require analysis of host factors that escape laboratory analysis. The immune system is influenced by nutrition, age, sex, and hormonal factors (21, 85). Developmental primary and acquired immune deficiencies also have major effects on host interactions with microorganisms and viruses. The spectrum of these relations is controlled also by genetic factors, regional diets and customs, altitude, and other geographical factors. Indeed, immune competence of a host may be influenced by psychodynamic and psychosocial factors (50, 66). Thus, animal and in vitro models of host-microbe interactions are always incomplete and fail to mimic the ecosphere sufficiently.

PARASITISM

Reservations about Koch's postulates are not new. They have concerned scientists since the introduction of the concept of pathogenicity. Theobald Smith (78) formulated an approach to the role of the microbial world in health and disease with his consideration of the host-parasite equilibrium and his explanation of parasitism in general. To Smith, parasitism is "a universal biological process that evolved from the predatory struggle for food and, therefore, represents the normal interdependence of all living things." Parasitism and the meaning of the word parasite should not be interpreted to connote the present vernacular interpretation of the terms. Instead, parasitism represents the Aristotelian perception of the interactions between very complex living and, therefore, metastable systems that bear on the life and survival of all living forms on this planet. It may not be a desirable portrait of humans, but we are, along with other animals and most microorganisms, chemoorganotrophic heterotrophs, organisms that require prereduced organic molecules to obtain energy and the materials we cannot synthesize. Predation, defined by Smith (78) as the search for food, is inherent in all nonphotosynthetic forms, and life for these representatives is possible only if they can exploit, that is parasitize, other living creatures. Human beings participate fully in this biological process, taking and giving their share. To paraphrase Savage (65), with tongue in cheek, the human body consists of approximately 10^{13} cells while our very own microbiota may reach 10^{14} to 10^{15} individual microbes. Who then parasitizes whom? Clearly, there is a most involved and necessary, even special, relationship between all so-called higher forms and their microbiota, a relationship vital to all components of this interaction.

Smith's broad definition of parasitism permits examination of infection from a dramatically different point of view. The study of plant and animal ecosystems (78) led him to suspect that disease reflects a disturbance in the host-parasite equilibrium. While he lacked much of the information now at our disposal, he proposed that infection results from chance encounters and entry of "foreign" and possibly free-living microorganisms or viruses. The manner of transmission is of little consequence. The important concern is the actual encounter and access of a microbial form not previously resident in this host or in a particular anatomic site. This chance parasite can express properties that may prove injurious to the host but that are not newly synthesized in response to the host environment. Instead, these factors play an integral part in that organism's survival under any circumstances or represent its ability to adapt to a situation requiring this type of response. The emphasis here is on the chance expression of microbial properties that permit the organism to tolerate the host environment and express characteristics that secure its niche and allow the microorganism to multiply. The host's damage, therefore, is governed by chance and represents the parasitism category that Smith (78) termed antibiosis. Smith also addressed beneficial parasitism consisting of mutualism, symbiosis, and commensalism, a state of peaceful coexistence in which the parasite does not monopolize nutrients required by the host.

ROLE OF THE HABITAT

Alexander (1), concerned with the relationship between a species and its environment, noted that each environment challenges the nutritional, physiological, and morphological capabilities of the species. Habitat ecology must be considered to clarify the circumstances that govern the appearance of infection. In nature, such consideration must include an assessment of the interactions between the different microbial species in a given habitat, the specific and the collective activities of the organisms, and an environment that is equally active and varied in response to the microbial presence. When any one microorganism in a human or animal ecosystem attempts to modify its surroundings, a dynamic community may be selected with repercussions for the anatomic site and the companion organisms. Colonization of the site and subsequent sequential colonizations result from microbial modifications of the microenvironment achieved by the original colonizers.

The microbiota of the human body can be divided into two groups (1). The intimately associated microorganisms that remain constant in and on their usual anatomic sites and which may play a role in the host's normal functions are autochthonous microorganisms. The second group, composed of microbes accidentally acquired from air, food, and other contacts and with the capacity to accumulate on exposed body surfaces, are termed allochthonous. Generally, this group cannot "cope successfully with the biological stresses or the abiotic factors in the new surroundings and they are ultimately eliminated" (1).

The various anatomic sites of the human body, suitable for microbiological habitats, display overlapping boundaries and are subject to microbial and host variations, some of which are predictable and others of which may be catastrophic. These complexities make analysis difficult but reinforce the concept that the presence or the recovery of a particular organism may not always be equated with disease. Indeed,

the niche of a microbial species, rather than its physical location, may describe its unique function within a habitat. This niche allows the expression of the biochemical, nutritional, and physical functions of a particular species. Nevertheless, different species usually share the same niche, while members of the same species may occupy different niches in dissimilar habitats. This explanation accommodates the notion that a microorganism can be pathogenic in one host and not in another or that harmlessness in one niche in the host does not obviate pathological changes in a different environment in the same host. Yet it must be reiterated that the harmful activity of the microorganism remains a response to the conditions in a select habitat or niche; its harmfulness is a byproduct of the influences exerted in the microenvironment.

HOST-PARASITE EQUILIBRIUM MODULATIONS

In his efforts to regard infectious disease as a natural disturbance of the host-parasite equilibrium, Smith (78) refuted Koch's denial of biological variation within a given microbial species. While Smith excuses Koch's insistence as a reaction against the unsubstantiated claims and lack of technique of contemporary investigators, he suggested that the accidental parasite, capable of producing disease, must differ in some fashion from the nonpathogenic member of the same species. He thus forecast the many more recent discoveries that demonstrate the role of lysogeny, plasmids, episomes, etc., as illustrations of this difference. The initial doubts raised by Smith (78) engendered theoretical support from Dubos (25) and Burnet (12), but the recent observations of the aforementioned hospital-acquired infections, legionellosis, and especially the opportunistic infectious complications of the human immunodeficiency virus disease force us to reconsider the mechanisms, if not the meaning, of pathogenicity and virulence.

The normal microbiota, this consortium of bacteria, viruses, fungi, and protozoa (43), has a profound effect on the health and disease of all living forms. Its beneficial activities are ignored most of the time, but studies with experimental animals (27) and certainly the investigation of herbivore nutrition (11, 38) stress dependence of higher forms on the microbial activities.

Even a cursory summary of these relationships is outside of the scope of this essay, but Rosebury (63, 64) pointed out that the so-called sterile areas of the human body are frequently, if only transiently, contaminated by members of the normal microbiota during normal daily activities. This observation reinforces the notion that some mutualism or symbiosis, aspects of beneficial interdependence (52), exists in the human situation and that this normal but dynamic collection of microorganisms must play an important role in protecting exposed body surfaces from colonization by strangers. This peaceful coexistence between humans and their microbiota may be beneficial or indifferent. It complicates the consideration of pathogenicity since some of the ordinary members of this microbial group are amphibionts (63), i.e., "sometimes" pathogens. The laboratory definition of such "turncoat" organisms, however, remains constant. Thus, the scientific advances of molecular biology are as yet unable to define in chemical or physical terms the microbial expressions of pathogenicity or virulence. One would expect such properties to fall within the limits of a select group of molecules, universally expressed by pathogenic and virulent organisms. Besides the toxins, produced as part of normal microbial activities but in the domain of parasitism, there are a number of microbial expressions, such as the colonizing factors, presently defined as virulence factors. Colonizing factors suffer the same shortcomings as the toxins in terms of their pathogenic designations (see below). Pathogenicity and virulence require more than just an organism with the potential to do harm; the manifestations of harmfulness demand a singular, particular host unable to respond in the normal fashion to microbial activities at that particular time and in that specific environment. In other words, it is a specific host that is the major determinant of the overt clinical manifestations of infectious disease.

THE MICROBIAL WORLD

The relationship between the human host and the microbiological atmosphere in which the host exists represents the interaction of two very complex systems. Unfortunately, many significant factors that control this interplay are unknown or incompletely studied, especially host responses at the cellular and subcellular levels. Although interest in these aspects has grown recently, the roles of genetic, environmental, nutritional, and psychological factors remain largely unknown. Instead, explanations for infectious disease have been sought through investigations of the microbial component of the host-parasite interface, an approach with many shortcomings. At this point, however, the microbial world as such requires attention since the archaebacteria, procaryotes, and protista are indispensable agents that permit life to exist and continue on this planet. Microbial forms are ubiquitous on the earth's surface, on plants, and in water and constitute a seemingly inexhaustible reservoir of microorganisms capable of interacting with the human host. A closer look at the physiological niches of certain archaebacteria and procaryotes indicates a preference for environments devoid of most if not all organic constituents. These forms, especially the chemolithoautotrophic and photolithoautotrophic organisms, lack the ability to adapt to the human biosphere. They can be regarded as xenobionts, microbes incapable of tolerating the hospitality of animal or even plant structures. In contrast, the very considerable numbers remaining in the natural reservoir can tolerate, or even prefer, the niches and habitats provided by the activities of other living organisms. Their entry into the human biosphere occurs largely by chance. If the entry site is normal, i.e., skin or mucous membranes in contact with the environment, the particular microbial and host conditions extant determine the survival and the future role of the newcomer. Accidental or traumatic entry of "unusual" microbes to usually sterile anatomic sites can result in disease, but this consequence depends on the microbial number, the host's health and experience with the newcomer or closely related representatives, and the consequences and care accorded the individual patient during the posttraumatic period. If introduction of natural reservoir microorganisms involves the usually exposed host surfaces, the newcomer must overcome a series of challenges if it is to become even a transient resident. The microorganism must find a means by which to adhere. This could be the usual microbial community to which the new arrival could attach because liganding structures are complementary to the residents' receptors. The newcomer must also be able to compete for nutrients and, if aerobic, oxygen and to resist the end products of the microbial consortium (17). These end products are the result of metabolic and physiological activities and may include bacteriocins, bacteriophages, and metabolic products potentially harmful to any microorganism seeking a haven in an established community. Similar

considerations attend the actual attachment of a newcomer to a host surface. To gain its initial success, a new arrival must be able to find conditions it can tolerate while not stimulating a sequence injurious to its survival at this particular site.

The impetus for newcomers to establish themselves on human surface structures results from the unintentional and intentional responses of the host and the usual colonizers of human surfaces. Within hard-to-define limits (85), healthy humans harbor a select group of microorganisms. We know most about the procaryotic members of the group, although fungi, protozoa, and viruses are obviously involved. The exact role of viruses in this context requires further evaluation. The normal microbiota has been shown for several anatomic loci to be selected on the basis of their exopolysaccharides and the host organ or tissue-specific glycocalyces (14, 18, 30). In such locations microbes are not harmful to the host and often serve a beneficial purpose. Nevertheless, the resident microorganisms exploit local injury or disease situations that impair the general capacity of the host to contain the normal microbiota (63), the condition described as amphibiosis. Most members of the normal microbiota are amphibionts or "sometimes" pathogens, usually harmless but endowed with the potential to expand their domain when their host is disadvantaged by illness.

The normal microbiota is a very dynamic congregation (14) that reflects local habits and natural environments. Travel, changes in food habits, etc., alter the constitution of the microbiota. The most far-reaching effect is caused by the medical and nonmedical use of antimicrobial agents during periods of anti-infective therapy or as a result of antibiotic addition to foods including vegetables and meat products, cosmetics, over-the-counter medications, animal feeds, etc. Susceptible members of our intimate microbiota are replaced by resistant individuals of the same or totally different species. When antibiotic therapy is needed and used, the resistant minority of the resident microbiota rapidly achieves majority status. In addition, new, antibiotic-resistant microorganisms that now gain access to a particular host stem from the reservoir in nature. The major attributes that permit their involvement in disease complications are antibiotic resistance and the host's impaired immunity. Despite their ubiquity, these microorganisms cause disease almost exclusively in the hospital setting; rarely are patients admitted with infections caused by resistant opportunistic organisms involved in hospital infections that may be called nosocomiants. The large quantities of antibiotic agents used in medical facilities and the practice of aerosolizing injectable antibiotic drugs into the hospital environment to assess the patency of needles place institutions at the very apex of a selective pressure pyramid for antibiotic-resistant microorganisms. The many water reservoirs required for modern therapeutic devices, the numerous decorative potted and cut flowers, vegetables, personnel, visitors, and air exchanges all serve as vectors to introduce these microorganisms (42). Medications, soaps, sinks, and inadequate sanitary practices (40, 44) also help to establish, nourish, and disseminate these offenders that plague patients whose immunity has been diminished by disease or therapy. Unfortunately, these organisms may complicate the recovery of especially those individuals who have benefited the most from the most recent advances in medical science. The circumstances result in a division of the microorganisms involved in human disease production, namely, those involved in community-acquired disease and those involved in hospital-acquired infections. The different attributes displayed by these distinct populations suggest a spectrum of microbial activities that may lead to overt disease and emphasize the determinant role of the host's immunity in allowing the expression of "pathogenic" microbial attributes.

We can then regard the microbial pool in nature as composed of organisms I have called peribionts and xenobionts. Peribionts may be defined as organisms capable of tolerating plant, animal, and human biospheres. They may become nosocomiants; they may also establish themselves as amphibionts or "sometimes" pathogens either directly from the peribiont level or through the nosocomiant stage (42). Either stage can progress to the pathogenic level if a particular host's susceptibility or general immune status permits the expression of microbial harmfulness. This orientation precludes a surprise reaction when hitherto unknown organisms or viruses, in addition to the many we consider harmless commensals or saprophytes, are involved in overt infections. The interface of each higher life form and the microbial world constitutes a spectrum of activities by each very complex component that includes a level of harmfulness we regard as disease. But even this stage is a natural part of the continuum of these interactions and does not necessarily reflect an abnormality in a philosophical sense. It does imply that our aforementioned anthropocentric preoccupation (41) with health, food, and wealth has left us without an appropriate understanding of microorganisms and their relationship one to the other and with all other living forms, including humans.

ROLE OF CHEMOTAXIS

In accordance with Theobald Smith's view, the first encounter of a microorganism in a host often is purely accidental, an assumption shared by and extended even further by Monod (59). Subsequent events are governed by factors listed earlier. Rather recent findings suggest that some microorganisms influence the ensuing sequences by actually selecting host environments that provide desired nutrients. The same mechanisms may guide a bacterium away from harmful or undesirable areas. Koshland's (49) evaluation of bacterial chemotaxis with *Salmonella typhimurium* and *E. coli* demonstrates the presence of constitutive and inducible receptors in the plasma membrane or periplasmic space of these bacteria. The bacterial receptors, under genetic direction (9), combine specifically with compounds that range from oxygen to pentoses, hexoses, select amino acids, and even phenol. The ligand-receptor complex triggers a mechanism in the cytoplasmic membrane that permits entry of methionine across the membrane. In the cytoplasm, *S*-adenosylmethionine is formed. This complex methylates the glutamates of a 70,000-dalton protein (46). The methylated protein carrier interacts with the insertion sites of the bacterial flagella. Usually, flagella join behind the bacterium and propel it by turning in a fashion resembling a ship's screw (56). However, methylation of the flagellar insertion causes the flagella to fan out, an action that leads the organism to tumble in the direction of a desired substrate or away from an undesirable one (8, 36, 68). When the membrane receptors are saturated, the process ceases, and the flagella return to their original configuration and propel the bacterium in the proper direction. This microbial sensing, akin to our sense of smell, takes advantage of the motion of peritrichously flagellated bacteria; how nonmotile or other motile organisms achieve this response is not yet known. Koshland's observations suggest that a sense of environ-

ment and the capability to respond are bacterial as well as eucaryotic properties that play a role in guiding organisms to a desired substrate and, by implication, to a potentially desirable surface on which they can be anchored.

DVLO THEORY

In the microbial universe the approach to another cell, especially giants such as mammalian cells, is not an easy task. We tend to forget our personal wonder at the unseen forces that agitate a bacterial suspension when viewed microscopically. We accept Brownian movement as the result of random solute collisions. It is but one type of obstacle microorganisms must circumvent in a progression toward a desired goal. Contact between cells is made difficult by the negative charge common to all biological cell structures. Since like charges repel one another, microorganisms must find the means to block this effect. Derjaguin and Landau, followed by Verwey and Overbeck, have studied this phenomenon of repulsion and attraction and explained the events in their theory of long-range attraction known as the DVLO (Derjaguin, Verwey, Landau, Overbeck) theory (22, 82). In summary, these investigators acknowledge that electrostatic repulsionlike charges of similar magnitude are found on all cells and are responsible for the inability of cells to contact one another easily. The basic constituents of all living cells are variations on the same themes. The basic molecules are identical, and the manner in which they interact is similar (54). Thus, carbohydrates, amino acids, purines, pyrimidines, inorganic constituents, etc., provide the material that, at a cellular level, follows the same architectural design but differs in complexity and differentiation as a reflection of genomic size (42, 54).

Parenthetically, there are obvious differences between the major divisions of living forms, but again these are variations on the same theme. Procaryotes are certainly more primitive than eucaryotes. Microorganisms, procaryotes and eucaryotes alike, differ from the higher forms by being acellular; i.e., they perform all essential functions of life within the confines of a cell-like structure. In higher forms, a division of labor is required that leads to specialization of cell functions accompanied by the inability of the individual cell to exist independently.

These similar, shared cell constituents create a universally negative charge on all cells and microorganisms and provide the electrostatic energy of repulsion. They also generate van der Waals-London forces that attract the cells to one another. These weak forces are the result of atomic and molecular vibrations that produce fluctuating dipoles; the electromagnetic interactions between atoms and molecules of similar fluctuation frequency produce an attractive force. Since the energy of repulsion declines more rapidly as the distance between cells increases, the long-range attraction forces permit cells to approach one another. Unfortunately, at very close range, the repulsive forces are the stronger; bacteria cannot muster the kinetic energy to deal with this repulsion. While the shape of the organism and of the host's cellular site may decrease this effect (curvature of each or both decreases the attraction and repulsion), the ability to adhere in the turbulent environment of most exposed human surfaces would disturb such connections (39).

MICROBIAL ADHESINS

Microorganisms, especially bacteria, have learned to overcome this final gap that separates them from cellular and even inanimate surfaces by producing various structures that bridge the final separation (58). Attachment is facilitated by the extremely small radius of adhesins that may be able to react with appropriate receptors on the mammalian cell surface in spite of and still influenced by the physical forces that are present. We must remind ourselves that clinical laboratory studies of microorganisms are subject to the pure-culture technique; we separate the microbial forms in a clinical specimen under very unnatural conditions and propagate them in a nutrient-rich environment that avoids the challenges found in nature. In the protective atmosphere of the test tube or petri dish, bacteria need not expend energy to produce adhesins, at least not to the degree required for survival in nature. Obviously, laboratory-cultivated variants of the organisms that once colonized or infected our bodies may not express a number of attributes required for survival in the natural setting. These differences led to the conclusion that bacterial adhesins are virulence factors, required for the expression of bacterial harmfulness. While this definition may pertain in select instances, it is once more a reflection of our collective, aforementioned preoccupation with disease, rather than the biology and ecology of the microbial world and its universal interactions with all living forms.

Despite the selective effect of laboratory domestication, there is good circumstantial evidence that chromosomal or plasmid-mediated abilities to select particular types of hosts or even specific host tissues or organs can be expressed by variants within a bacterial species. Several factors underlie these in vitro observations (15). Convincing evidence has been presented (19) that, in nature, microorganisms, and especially bacteria, prefer to live in microcolonies, structures that provide appropriate nutrition in a niche capable of entrapping soluble nutrients and protecting the microorganisms from harmful environmental substances such as chemicals, end products, surfactants, antibiotics, and antibodies. Individual swarmer or explorer organisms are released periodically and, if they encounter favorable conditions, establish additional colonies that may include other microorganisms. Such advantageous habits lead to the establishment of a consortium consisting of different microbial species and genera. The protective exopolysaccharides can join and contribute to the safety of the consortium. A second equally significant attribute of procaryotic and eucaryotic cells is their ability to modify the physicochemical microenvironment by the production of specific complementary adhesive structures that permit interactions. These adhesins, under the genetic control of the individual organism, mediate attachment once a close approach between the representative surfaces is possible. The mammalian host can, thus, attract a usually harmless surface microbiota that prevents attachment of microorganisms capable of damaging the surface or the host. At the same time, microbial activities may provide additional benefits such as host-useful end products or activities. The microorganisms benefit by exploiting the host surface as a holdfast in keeping with their preference for microcolony and consortium formation.

The evidence for this mutual selection of host or tissue/organ derives from several studies summarized by Christensen et al. (15). *N. gonorrhoeae* will adhere only to the human oviduct, not to that of rabbits, pigs, or cows; enterotoxigenic *E. coli* isolated from humans with disease adhere specifically to human ileal cells, cells ignored by enterotoxigenic *E. coli* from pigs, calves, or rabbits. Cell preparations from the tongue of humans or rats will allow adhesion by oral streptococci and corynebacteria derived from the corresponding host. *Shigella flexneri*, capable of

producing diarrhea in humans and guinea pigs, will adhere more strongly to guinea pig colonic epithelia than to the same cells from rats, rabbits, or hamsters. Similarly, *Bordetella pertussis* prefers human respiratory mucosal cells to those from other mammals, while *Bordetella bronchiseptica* selects animal mucosal cells in preference to the human variety.

Christensen and co-workers (15) cite various examples of the role of host susceptibility to bacteria. *Pseudomonas aeruginosa* attaches preferentially to the buccal epithelial cells of cystic fibrosis patients; *Staphylococcus aureus*, to nasal mucosa cells of carriers; *Streptococcus pyogenes*, to the pharyngeal cells of rheumatic fever patients; and *E. coli*, to vaginal, buccal, and urinary epithelia of patients with frequent urinary tract infections. Tissue tropism is also manifested. *Neisseria meningitidis* adheres to nasopharyngeal cells in preference to cells from other sites such as buccal, urethral, bladder, or anterior nares epithelia. *Pasteurella multocida* from a rabbit nasopharynx adheres to nasopharyngeal epithelia more strongly than to ciliated respiratory epithelial cells or various mammalian cells in culture. *Staphylococcus saprophyticus* preferentially combines with cells of urogenital origin in comparison with cells from skin or buccal mucosa. *Streptococcus pyogenes* from skin adheres better to skin cells than those from the oropharynx in contrast to streptococci isolated from the throat.

These interactions between microorganisms and hosts and the mechanisms that govern these events at the cellular and subcellular level underline dramatically that these interactions are not one-sided but that a mutuality, a complementariness, must pertain. In other words, a microbe of and by itself can be only a transient presence in a host unless host factors provide it with a haven, site, or niche where attachment can occur regardless of subsequent events, be they beneficial, harmful, or equivocal. The idea of pathogenicity as a property of the microorganisms or virus is, therefore, incomplete if it does not bring to this consideration the significant, determinant role of the host. While our collective ignorance still does not explain acceptably all of the forces at work that promote the undesirable, disease-associated attributes of host-parasite interactions, we must recognize that pathogenicity is the result of shared contributions by both participants. Also, we must admit that for a century we have ignored almost completely the host's contributions to this series of natural interactions that include, as a small segment, the expression of harmfulness. The study of microbial etiological agents suited the simplistic approaches that reflected primitive technology, nurtured by philosophical anthropocentric attitudes. The primitive technology which still represents the majority of our activities in the clinical laboratory is now in the process of yielding to modern approaches in the analysis of the microbial participants and is beginning to permit at least an appreciation of the host's contributions.

INFECTION AND GLYCOCALYCES

The recent advances in understanding the immune responses of the human host are too vast to summarize here. We must accept that a particular host's total immune response plays the determinant role in the success of any microorganism as a pathogen (42). We can regard infectious disease as a consequence of active host selection of etiological agents or an impairment of one or more normally active functions that at a particular moment are not expressed for one or more of the reasons that embarrass the immune system. In contrast to presence on a host surface, once entry of a microorganism has been achieved, tissue involvement and cell destruction ensue in the impaired individual, resulting in generalized disease and, possibly, demise. Only appropriate therapy can slow the process sufficiently to permit the host to interrupt this progression. We have been satisfied with this overview of events (21, 85), bolstered by our knowledge of the numerous nonspecific and specific components and constituents of the immune armamentarium (28). We may include prostaglandins, Hageman factor, human leukocyte antigens, lymphokines, etc., in this view and have some confidence in our appreciation of the details that govern the success or failure of therapy. A series of advances in several, rather unrelated areas of biology have produced the need to investigate the role of host receptor and liganding molecules that may participate in many of the events that constitute the host-parasite equilibrium and its disturbances. Despite the early stage of comprehension, it has become evident that procaryotic and eucaryotic cellular activity, controlled by deoxyribonucleic acid (DNA), requires some input or signal that recognizes the cell's or organism's location, energy and nutrient requirements, and, in a sense, functions and procedures. Aware of antigens on the mammalian membrane surface, we know that hormones and many other essentially chemotactic mechanisms control a number of cellular responses and activities. Emerging from a massive collection of individual observations is knowledge of the presence of a code on the surface of all cells. This code, described as the eyes, nose, and ears of DNA, is as critical as the genetic code, is present on the surface of each cell, and specifies the cell's function while directing its interaction with all other cells and creating an almost inconceivably intricate cellular communication system (10, 67).

Thus, cells possess their own identification code and the capabilities to interpret the codes of other cells. A great deal remains unknown about the nature of this membrane code, but it appears concentrated in the glycocalyx, the polysaccharide projections anchored to proteins or lipids of the cell membrane. The mammalian glycocalyx code consists of the following seven carbohydrates: glucose, galactose, mannose, fucose, acetylglucosamine, galactosamine, and sialic acid. Obviously, the several functional groups on each of these sugars permit a vast number of combinations with its companions that can be modified further by the type of glycosidic bond, forming either an alpha or a beta glycoside. This membrane code exceeds by far the possible combinations we accept for the bases in DNA which connect to one another in a linear fashion. The seven simple sugars can be arranged to form innumerable unique structures, usually consisting of no more than five of the seven carbohydrates. These different codes on different host cells aid in governing interactions among themselves by virtue of specific receptors and with microbial representatives and their many particular expressions and modalities of communicating.

BACTERIAL SURFACES

Gram-Positive Bacteria

Bacteria, the most intensely examined group of microorganisms, have developed a variety of approaches to exploit the varied receptor site of glycocalyx-coated host surfaces. The bacterial mechanisms studied so far indicate that methods of adhesion reflect the ultrastructure of the organisms

and that the organisms can be classified on the basis of the Gram stain with but a few exceptions. The gram-positive bacteria produce a series of projections that reach beyond the confines of the cell wall and even capsules when present. The most common mechanisms for their attachment are the teichoic acids, the ribitol or glycerol phosphates anchored by phosphodiester bonds to polyol residues (83). When phosphate is limited in the bacterial environment, some gram-positive bacteria produce teichuronic acid, an equimolar mixture of galactosamine and D-glucuronic acid, linked directly to muramyl residues through a phosphodiester. The synthesis of these secondary polymers takes place prior to cell wall assembly. They are attached to the peptidoglycan units before they are assembled into the cell wall structure, with the exception of staphylococcal protein A, bound to peptide amino groups in the peptidoglycan. The various proteins associated with the outer surface of gram-positive bacteria are not covalently linked to the cell wall. The M proteins and other protein surface structures found on many streptococci are associated with the cell wall in a manner not yet understood (5). The most significant cell wall projections of the gram-positive bacteria, the lipoteichoic acids, are amphiphiles, or compounds that display hydrophilic and hydrophobic properties. The hydrophobic nature of these projections provides a means of interacting with hydrophobic domains present on host cells. While the variously substituted polyglycerols or ribitols are hydrophilic, the phosphorylated monoesters at the end of the chain become hydrophobic by either glycolipid or phosphotidylglycolipid substitution. These lipoteichoic acids interact ionically or hydrophobically with proteins of the host cell. Deacylation of the lipoteichoic acid lipid moiety prevents hydrophobic aggregation and protein interaction (62). Some gram-positive bacteria produce, instead of or in addition to lipoteichoic acids, polymers containing lipid. For example, *Micrococcus* spp. produce a lipomannan consisting of 50 to 70 D-mannose molecules, some of which are succinylated, that covalently link a glycolipid at one end. Actinomycetes produce heteropolysaccharides with fatty acid substitutions as a means of attachment to eucaryotic cells. Lipoteichoic acid is known to facilitate the staphylococcal binding to buccal epithelium and the adhesion of *Streptococcus mutans* to dental surfaces. The streptococcus not only exploits the ability of hydroxyapatite to adsorb lipoteichoic acid but also interacts with its surface enzyme, glucosyltransferase. This combination of lipoteichoic acid and enzyme forms the foundation of dental plaque (15).

Many of the extracellular proteins of gram-positive bacteria are hydrolytic enzymes, often suspected to be important agents of pathogenicity. Most of the time these enzymes, when purified, were not able to mimic the symptoms of the specific disease associated with the etiological agent. Exceptions, of course, are the various extracellular protein toxins of gram-positive bacteria. The synthetases that lead to glucan and fructan polymers produced by *Streptococcus mutans* and *Streptococcus sanguis* may be regarded by some investigators as aiding in the harmful aspects of these bacteria subject to conditions in the host environment. A number of these organisms produce immunoglobulin A proteases that tend to lessen the inhibitory effect of immunoglobulin A. The extent of damage ascribable to these enzymes is not known. The bacterial surface in nature displays a geometric array of glycoprotein subunits, the S layer. The varying patterns of the S layer are formed electrostatically on the bacterial surface and in some cases may involve divalent cations in its assembly. The exact role of these structures is still unknown. If they display multiple sugar-binding sites, the externalized proteins and glycoproteins of the gram-positive bacterial surface may act as lectins. Demonstration of lectin activity usually depends on the inhibition of the lectin-membrane reaction by including the mono- or oligosaccharide in the in vitro reaction mixture. Certainly, some of the bacterial fimbriae of the gram-negative bacteria fall within this group since they are proteins that bind bacteria to specific carbohydrates on animal cell surfaces. Carbohydrates are found only on the outer membrane of animal cells, combined with select amino acids and *N*-acetylglucosamine or *N*-acetylgalactosamine.

Gram-Negative Bacteria

The gram-negative bacteria confront their environment with cell wall arrangements of greater architectural complexity than the gram-positive organisms. The gram-negative peptidoglycan cell wall is considerably thinner than its gram-positive counterpart and lacks interpeptide bridges with the muramyl peptides usually linked through diaminopimelic acid and D-alanine. This cell wall is not closely associated with the cytoplasmic membrane but is separated by the periplasmic space, a fluid in equilibrium with environmental molecules of <1,000 daltons that can cross the outer membrane, isoosmotic with the cytoplasm and a reservoir for hydrolytic enzymes. The peptidoglycan and the outer membrane matrix protein form a hexagonal lattice over the peptidoglycan surface that links the cell wall and outer membrane ionically. A small lipoprotein of the outer membrane is covalently attached to the carboxyl terminus of a number of diaminopimelic acids in the peptidoglycan.

The outer membrane of the gram-negative bacteria carries molecules, many of which may be "seen" by the immune system of the host. The surface of the organism or virus interacts with the host and, as such, can be the purveyor of host-harmful activities. Pathogenicity and virulence differences between organisms are most likely to be expressed at that time. As mentioned earlier, no group(s) of compounds has been identified that imparts host-detrimental properties to any organism other than the protein exotoxins. These molecules, many of which are enzymes, fall within Theobald Smith's category of "accidental" harmful attributes, compounds that serve a purpose for the organism but prove to be deleterious to the host when liberated in the host environment (42). A number of surface structures of gram-negative bacteria have been designated virulence factors that are equally accidental expressions promoting advantages to select members of a species. *E. coli* is the most intensely studied of all bacteria and serves as the basis for the numerous inferences concerning the behavior of gram-negative organisms. In excess of 20 different polypeptides are found in its outer membrane, a modified version of the universal structure surrounding all cells. The major proteins encountered are matrix protein, lipoproteins, and the proteins that constitute the porin channels. Numerous minor proteins function, perhaps not exclusively, as receptors for such different purposes as bacteriophage attachment and transport of select nutrients including vitamin B_{12}, iron, nucleosides, and certain carbohydrates. They may also participate in forming a barrier to hydrophobic substances such as detergents and certain dyes (61). The outermost surface is adorned with glycocalyces consisting of species- or strain-specific homo- and heteropolysaccharides. These may be glucans, levans, or sialic acid polymers. Others

consist of repeating units of several carbohydrate residues. Certain of these sugars are peculiarly bacterial. Uronic acids, pyruvate, and other compounds can also participate, creating a very complex surface structure that we recognize as the somatic antigens of these bacteria.

Bacteria, especially the gram-negative ones, display a variety of additional surface appendages that arise on the surface or project through the cell wall. In addition to flagella, they display various adhesins, structures that permit bacteria to cling to inanimate surfaces or those of plant or animal cells, other bacteria, or microorganisms. Isaacson (39) refers to these appendages as pili, while Beachey (4), Freter (32), Isenberg and Balows (42), and Jones (45) differentiate between pili which are the plasmid-determined fertility factors and fimbriae. The proteins that comprise both pili and fimbriae are variations of the pilin molecule that range from 11,800 daltons for the F pilus to 64,000 daltons for *Actinomyces viscosus* fimbriae. Some of the fimbriae carry phosphate, sugar, or phospholipid substituents. The fimbriae of many different species and genera display regions of amino acid sequence homology but lack antigenic cross-reactivity. Different types of fimbriae may be encountered on some bacteria, suggesting that organisms bind to mammalian cells by more than one mechanism. Comparatively little is known about the specific mammalian receptor sites for the various fimbriae; some fimbriae are produced constitutively such as the type 1 fimbriae of *E. coli*, and their adhesion to mammalian cells can be blocked by mannose, suggesting a role for this sugar on the mammalian receptor (30, 31). Many other fimbrial types are not inhibited by mannose, such as the fimbriae of *N. gonorrhoeae*, the *Enterobacteriaceae*, and pseudomonads. These include K88, K99, CF1, and CF2 of *E. coli*, the fimbriae of *Klebsiella*, *Pseudomonas*, and *Enterobacter* spp., and the P fimbriae of uropathogenic *E. coli*. For their attachment to epithelial cells, the latter fimbriae depend on P blood group types, determined by globoseries glycolipids that display a galactose disaccharide, α-Gal-1→4-β-Gal (53). The attachment of K88 fimbriae to host cells can be prevented by β-D-galactose. These specific receptor definitions suggest that the fimbriae act in the manner of lectins, proteins, or glycoprotein structures with multiple sugar-binding sites. In view of the frequent association of different bacteria in a colonizing consortium, receptorlike substances may also be found on microbial surfaces; mucin and Tamm-Horsfall protein also offer the opportunity for adhesion. The mechanism for preferable invasion described by Goodpasture (35) has not been explained on the basis of receptor-ligand interactions. Using the chicken embryo model, Goodpasture demonstrated that *B. pertussis* invaded ciliated bronchial epithelium, whereas *N. meningitidis* attacked the meninges and *Streptobacillus moniliformis* attacked the joints of these embryos. Similarly, the attraction for *Brucella abortus* (77) of erythritol in the ungulate placenta increases one's suspicion of mammalian tissue selectivity based only on specific receptors. Fimbriae may be the ligands that place bacteria in specific anatomic loci where under most circumstances they act as normal microbiota, possibly providing mutual benefits. So far, few host-parasite relationships have been analyzed with emphasis only on fimbrial adhesion. Fimbriae resembling type 1 can be demonstrated by hemagglutination reactions and are encountered not only in *E. coli* but also in *Enterobacter* spp., *Shigella flexneri*, *Klebsiella* spp., *Serratia marcescens*, and many serotypes of *Salmonella* spp. They attach readily to animal and plant cells and induce pellicle formation in static broth cultures, and their reaction with mammalian receptors may or may not be inhibited by mannose. Type 3 fimbriae, found in *Enterobacter aerogenes* and *Serratia marcescens*, promote adhesion to fungal and plant surfaces, cellulose fibers, and glass but do not react with animal cells. *Proteus mirabilis*, especially, displays type 4 fimbriae that are mannose-resistant hemagglutinins and may function in pyelonephritis (69). Another type of fimbriae may permit this bacterium to adhere to renal pelvic epithelium (45). *Vibrio* spp. possess fimbriae and supplement their adhesion to mammalian epithelial cells with modified flagellar structures, capable of hemagglutination. Gonococcal fimbriae play a role in the bacterial adhesion to their host target cells and account for their agglutination of human erythrocytes. Other neisseriae display different types of fimbriae or none. *B. pertussis* attachment to epithelial cells is mediated by high-molecular-weight fimbriae. Jones (45) proposed the term fibrillae for nonprotein surface adhesins that include the various teichoic and lipoteichoic acids of streptococci and staphylococci, the polysaccharide-rich projections of the lactobacilli, and nonfimbrial adhesion of gram-negative bacteria (29, 34).

EXOPOLYSACCHARIDES

The microbial adhesins, thus, play an important role in attaching microbes onto specific surfaces, a role that must, in some as yet undefined fashion, provide advantages for the organism. The preference of organisms for establishing microcolonies will lead different species to use the microcolony as their holdfast as long as the microbial receptor-ligand interaction can ensue. Undoubtedly, some selection process is operative at this level as well, and one would expect mutual advantages from these microbial associations such as sequential utilization of one component's end products as nutrient for a neighbor. The surfaces of the human body harbor dynamic polymicrobic populations. The turbulent physical activities in these anatomic loci require that the adhesin-mediated microbial attachments be further secured. Since bacterial glycocalyces of varying rigidity and complexity (16, 18) are constantly shed into the intimate microbial environment, the colonizing consortium can be cemented in place by the interaction of these diverse carbohydrate moieties (80). A large variety of complex organic rearrangements produce fibrous, orderly, crystalloid structures that are quite hydrated but very hydrophobic on their outermost surfaces (6). The exopolysaccharide cement protects the microbial biofilm on host surfaces against physical dislocation. More significantly, the exopolysaccharide cannot be penetrated by antibiotic agents in sufficiently high concentrations to affect the bulk of the microbial colonizers. Mammalian enzymes are incapable of breaking the β-glycosidic bonds that link many of the carbohydrates, and phagocytes are unable to breach the exopolysaccharide barrier. The microbial consortium and its individual residents are, therefore, truly outside the human body; i.e., they are not recognized by the immune system as long as they remain within the confines of the consortium. The lack of host immune recognition of the individual colonizing microorganism that does not venture beyond the safety of its niche underlines the need to distinguish colonizing and infecting microorganisms. Certainly this failure of the host immune system to recognize members of the microbial surface communities explains the ability of amphibiotic organisms to take advantage of the host when disease or injury permits microbial penetration below surface tissues. The immune

system encounters these particular bacterial species or variants only after they have penetrated or at least have left the safety of their exopolysaccharide fortress. The immune system reacts to these explorer organisms as newly encountered microorganisms and produces immunoglobulins that recognize the outer surface components of the individual microbe, which differ considerably from the exopolysaccharide cement encountered in the colonizing consortium. Thus, despite a long association between bacterial species and the human surface, the host immune system may not be acquainted with some of the organisms that constitute the so-called normal microbiota. If such bacteria succeed in finding a favorable attachment site in deeper tissues, they may well begin to form microcolonies, surround them with exopolysaccharide layers, and defy the immune system once again. The occasional explorer microorganism that may be liberated from such internalized microcolonies then represent an exacerbation of a chronic infection in certain tissues.

IMPLICATIONS OF THE CONCEPT OF PARASITISM

The intricate and intimate involvement of the microbial world in the activities of all living forms suggests that microbial involvement in disease production represents but a small segment of a vast continuum of interactions. Infectious disease, thus, becomes a developing series of events that requires the participation of the individual host and the microorganism. Certainly, the nosocomially involved bacteria, fungi, and protozoa demonstrate that it is the host and, to a degree, the environment that permit commensal organisms to complicate the recovery of patients. These organisms do not challenge or threaten nonpatients in the same environment or individuals in the community where the same organisms reside, albeit under not quite such intimate circumstances. We must examine the organisms traditionally regarded as pathogens and determine whether during disease initiation, the host exerts a decisive influence as well. The history of devastating epidemics would deny the host this determinant role, yet Burnet (12) concluded that the severity of the disease and survival were the result of the individual host's immune competence in the broadest sense of the term. It may be presumptuous to point once more to our determined preoccupation with microbial causes of disease, damage to our food supply, and the integrity of manufactured goods to the detriment of the broad scale of host-parasite interactions that include health, beneficial, and indifferent consequences as well. We also ignore the enormous reservoir of microbial forms in nature and are surprised invariably when they dare to intrude into our biosphere. Surely, the legionellae existed long before the human need for comfortable temperature provided a suitable vehicle for efficient dissemination of these organisms. Starr (79) recognized this deficiency in the attitude of microbiologists and physicians when he pointed out that some treasured convictions may distort our perceptions and misguide our efforts. He referred to disciplinal insularity as a consequence of increasing specialization that prevents those interested in medically important bacteria from knowledge or even interest in veterinary or industrial procaryotes. Starr explains that this attitude interferes with our recognition of ambilateral harmfulness, the microbial capability to be harmful in several settings such as displayed by the salmonellae to cite one example. Frequently, the same organism in different specialty settings is not designated with the same genus and species name and its true identity is not recognized until the organism is harmful in different host populations. Starr blames the persistence of these conditions on epistomological primacy, our overriding preoccupation with our own knowledge and concerns that does not admit understanding gained in areas outside of our interests. I believe that, in part at least, the latter attitude is governed by gnoseological paralysis (41), our unwillingness or perhaps our fear to know the extent, the limits, and the basis of our discipline. Most of all, we lack an appreciation of the dynamism of the microbial world that permits the microbial pool in nature to expand the dietary horizon of at least some of its members and to seek new opportunities to sustain their species. Time and again and over many human generations, this microbial reservoir in nature can contribute temporary microbial residents or colonizers to our intimate biosphere. If, by chance, an inadvertent change in the microorganism or in the human host permits the establishment of a relationship, the subsequently unpredictable reactions may be mutually beneficial, indifferent, or harmful to either or both. This scenario reflects the concepts of Theobald Smith but underlines as well the idea that overt disease manifestation is a mutual effort by the organism and the host. Besides the pathogenic category, the concept of parasitism contains the combinations already mentioned, namely, indifferent, mutually beneficial, or beneficial to one component while indifferent to the second. These established relationships need not remain static, but rather the host-parasite equilibrium can be and is disturbed repeatedly with consequences that may be beneficial, indifferent, or harmful. Hypothetically, the presence of an intracellular microorganism or virus provides the opportunity for genomic parasitism, a transfer or transposonlike interaction of microbial DNA and the genome of the particular parasitized cell (33, 81). Another possibility is the survival of a segment of microbial or viral genomes in the form of quasiepisomes. Extra-human DNA may then be duplicated whenever the mammalian cell divides. Its presence may never be noted or expressed. It is also possible that some subtle or more profound changes may occur immediately or at some later stage.

It would seem most likely that the microbial genome fragment survives passively unless and until an appropriate signal is received from outside the cell that initiates a host cell expression that differs totally from its normal activities and functions. One might dare to suggest that a viral or procaryotic genome segment in a mammalian cell that encounters one of the many carcinogens produced daily in the human intestine by the normal microbiota (23) is recognized by a corresponding receptor on the cell surface and could stimulate the viral or microbial genome in concert with select host genes to initiate a process akin to neoplasia (7, 51, 60).

Assuming that certain neoplasms, other organic diseases, and perhaps as yet unknown diseases represent genomic infections, it is disturbing that these disease expressions are not encountered with much greater frequency since potential signals or enhancers abound in the cellular environment of the host. A possible explanation for this comparative rarity may be gleaned from the considerations of the biological time sequence termed temporal serendipity. This term describes the precise nanosecond that allows the last required molecule to enter a multilayered physiological cascade with each component in the cellular machinery poised to move in a specific direction. Failure of the last compound to participate will lead to entirely different activities or metabolic efforts or both. While the precise function and activity of most cells remain a mystery, with multifarious processes

occurring simultaneously, we know that external signals, the entry of a nutrient or the elimination of an end product, proceed in an orderly fashion and with precision. A possible role of microbial genomic infection in such a sequence may not be totally impossible.

Pathogenicity and virulence are concepts with definitions that require correction. The host plays an undeniable role in the overt clinical manifestation of infection after exposure to specific microorganisms at a given point in time. Appropriate host receptor sites must be available; the immune competence may have to be embarrassed if microbial invasion is to succeed. Pathogenicity reflects the host-parasite equilibrium, governed by very dynamic ecological conditions. The application of an antibiotic drug directed against a likely etiological agent cannot guarantee successful elimination of disease, for impaired host factors will encourage other microorganisms, resistant to the therapy used, to continue to complicate the recovery of a patient. The degree of immune compromise often has a profound effect on the extent of infectious complications. But the change in definitions of pathogenicity and virulence has as a practical consequence the recognition that antibiotic therapy is not the final solution to the problem of infection. Admittedly, the developed countries are no longer subject to all of the epidemics and severe infections of earlier centuries. The incidence of infectious diseases is not apparent, however, from the statistics that list primary diseases without recording the role infectious disease complications played in prolonging the primary disease or contributing to a patient's demise. The development of successful control strategies for infectious disease must rest on the understanding of receptor sites, ligands, glycocalyces and their interactions, stimulation of nonspecific and specific host factors, host nutrition and diet, intermicrobial ecology, tissue or cell selectivity, and specificity for a particular microorganism. The onus of causality must be removed from the nonexistent shoulders of the microorganisms. Therapeutic efforts must be directed at restoring a tolerable host-parasite equilibrium.

Finally, we must recognize a very subtle and rarely considered aspect of our attitude toward the disease-associated propensities of the microbial world (24, 42, 70). Our view of infectious disease was generated by the giants of microbiology, Pasteur and Koch. In keeping with the prevailing attitudes of their time, they, their colleagues, and students perceived the interaction between microorganisms and the human host as a struggle consisting of microbial invasion and aggression, host defense mechanisms, antibodies, cellular defenses, etc. While our present views concerning infectious diseases have been modified to a degree, we continue to describe infectious events with the same terminology and, thus, remain subject to the psychological consequences of these terms. Noam Chomsky (2, 37, 55) implies in his theory of transformational grammar (psycholinguistics) that the use of terms such as antibodies and antibiotic maintains the prejudice associated with these words despite our conscious effort to deny such an association. The advances of biological philosophy, exemplified by Jacques Monod's *Chance and Necessity* (59), may help to reduce the anthropocentric attitudes of the 19th century and strengthen our appreciation that we and all living forms are part of a continuum of chance interactions. This broad interpretation of ecology represents the mood of our time, if not its philosophy. We must recognize this unity of biology superimposed on the unity of biochemistry (47) and the unity of molecular biology (54) and use ecological symbiosis as the means to improve and influence the interactions among living systems. Then, perhaps, all disease can be viewed as a natural sequence in a series of events, not determined by a particular presence possessed of harmful species or variant attributes but as just one segment in a progression of complex interactions. Emphasis on the Aristotelian aspects of this complex interdependency may establish the limits and define the numerous significant segments and sequences that pertain to parasitism and predation. Such understanding could provide a rational basis for the prevention and palliation of infectious disease.

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